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Interaction of Chlorpromazine with 2´-Deoxyguanosine-5´-monophosphate by PM3 Calculation

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Abstract. *Using the PM3 method, the interactions between chlorpromazine (CPZ) with 2'-deoxyguanosine-5'-monophosphate (dGMP) were examined. We obtained the optimized geometrical structure of each CPZ, dGMP and a CPZ-dGMP system in both aqueous-phase and gaseous-phase and investigated their geometric and electric changes and Force calculation. By Force calculation, three vibrations at 835, 800 and 737 cm^{-1} were assigned to the antisymmetric and symmetric P-O stretching vibration of PO_3^{2-} group in Z-dGMP, respectively. The vibrations at 889, 803 and 799 cm^{-1} due to the C5'-O-PO_3^{2-} stretching vibration shifted to their corresponding higher wave numbers, comparing to those of Z-dGMP alone.*

Phenothiazines have potential antitumor activity (1). The antitumor chlorpromazine (CPZ), one of phenothiazines, could bind strongly to DNA. Because of the chemical and structural similarities of CPZ with the carcinogenic acridine orange, benz[*c*]acridines and Nile blue (an antitumor phenoxazine dye) (2), it was intercalate into DNA in the same manner. A stabilized radical ($CPZ^{+\cdot}$) of CPZ was detected by electron spin resonance (ESR) when CPZ was added to DNA under pH 5.0 (3). Then, as indicated by Lerman's intercalation model, the helix axis of DNA is perpendicular to the aromatic molecular plane of CPZ (3). The complex formation of the CPZ-DNA system had non-Newtonian properties shown by viscosity measurement, and the Raman spectra of the complex showed a difference at 982 cm^{-1} because of symmetric P-O stretching vibration of the pO_4^{-2} group between dGMP alone and the CPZ-dGMP complex (4). The interaction of CPZ and dGMP in D_2O was investigated by proton NMR measurement (5). The CPZ complex showed a chemical shifts upfield, compared to CPZ alone. These shifts came from the stacking interaction of CPZ with dGMP, leading to a downfield shift of the proton signals of the 2'-deoxysugar. The comparison between chemical shifts in dGMP alone and a CPZ-dGMP complex, resulted in the aromatic protons of purine and CPZ shifting slightly upfield. This means that a CPZ-dGMP complex might be formed under this experimental condition (5). The purpose of this study is to represent the further interaction of CPZ with dGMP by parametric method 3 (PM3) calculation.

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Key Words: Chlorpromazine, dGMP, IR spectra, PM3 method, COSMO calculation.

Materials and Methods

Calculation. The PM3 method were performed with application of MOPAC 93 program (6). The geometries of CPZ and dGMP were optimized by using all geometrical parameters by Broyden-Fletcher-Goldfrab-Shanno algorithum in corporated in the program. In the case of dGMP, the energy profile of the stepwise rotation around the glycosidic C1'-N bond was calculated by PM3 method. The geometry of CPZ and dGMP in water by conductor-like screening model (COSMO) were compared to those in the gas-phase. The COSMO procedure generates a conducting polygonal surface around the system at van der Waal's distance. The key words used here were number of geometrical segments per atom (NSPA)=60, dielectric constant=78.4 at 25°C (water) and van der Waal's area was 147.18 square Angstroms ($Å^2$). For this calculation, the FACOM M1700 computer in the Josai University Information Sciences Center was used.

Results and Discussion

Electronic structure of CPZ. The geometrical optimization of CPZ in both of gas and water phases was carried out with optimization of all geometrical parameters with no assumption. The PM3 optimized geometries of CPZ in both phases were nonplanar (Figure 1). First, the optimized geometries of CPZ in water phase were similar to those of gas phase. All single C-C bonds and double C=C bonds of CPZ skeleton in two phases were calculated at lengths ranging from 1.393 to 1.407 Å, and from 1.386 to 1.387 Å, respectively; however, the lengths of C4a-C10a and C5a-C9a

Table I. *Normal coordinate analysis and assignments by Force calculation of CPZ from 3150 to 1700 cm^{-1}.*

	Phase				
	gas		water		
$\nu_{obs.}$ [a]	$\nu_{calc.}$ [b]	T [c]	$\nu_{calc.}$ [b]	T [c]	Assignment
	3133	0.03	3133	0.11	ν $(CH_3)_2$
	3131	0.08	3131	0.11	
	3075	0.57	3074	0.58	ν (C_7-H)
	3069	0.60	3068	0.60	ν (C_3-H)
	3061	0.07	3061	0.17	ν $(CH_3)_2$
3062	3060	0. 04	3060	0. 09	
	3060	0.37	3059	0.38	ν (C_8-H)
3020	3040	0.36	3041	0.05	ν (C_6-H)
	3040	0.34	3040	0.36	ν (C_4-H)
	3031	0.04	3039	0.05	
	3029	0.16	3039	0.34	ν $(CH_3)_2$
	3021	0.07	3019	0.11	ν (CH_2)
3011	3012	0.44	3012	0.47	ν (C_9-H)
	2997	0.35	3011	0.30	ν (CH_2)
3000	2957	0.04	2994	0.11	ν (CH_2)
2933	2947	0.17	2954	0.19	ν (CH_2)
2891	2928	0.13	2946	0.17	ν (CH_2)
	2891	0.01	2926	0.05	ν (CH_2)
	1783	6.00	2890	0.16	ν (C_8=C_9)+ν (C_{5a}-C_6)
	1772	0.51	2287	3.79	ν (C_6=C_7)+ν (C_9-C_{9a})
1589	1770	2.07	1783	1.00	ν (C_1=C_2)+ν (C_3=C_4)
	1755	0.61	1772	0.50	ν (C_2-C_3)+ν (C_{4a}=C_{10a})

a) Observed frequencies of CPZ · HCl (cm^{-1}).
b) Calculated frequencies by Force calculation (cm^{-1}).
c) T: Transition dipole.

Table II. *Normal coordinate analysis and assignments (1000-500 cm^{-1}) for dGMP and CPZ-dGMP system by Force calculation.* [a]

dGMP				CPZ-dGMP of Z-DNA	
dGMPof B-DNA	Assign-ment	dGMP of Z-DNA	Assign-ment		Assign-ment
975	δ (D-CH)	993	δ (G-ring)	964	CPZ
944	δ (D-CH_2)	964	δ (D-CH)	954	CPZ
936	δ (D-CH)	936	δ (D-CH_2)	950	CPZ
908	δ (G-NH_2)	890	δ (G-ring)	944	dGMP
878		866	δ (D-CH_2)	935	dGMP
864	δ (G-CH)	834	ν (PO_3^{2-})	932	CPZ
845	δ (G-ring)	829	δ (G-CH)	930	CPZ
835	ν (PO_3^{2-})	807	δ (G-NH2)	901	CPZ
800	ν (PO_3^{2-})	806	ν (PO_3^{2-})	891	CPZ
788	δ (G-NH)	759	δ (D(C-O-C))	889	ν(C-O-PO_3^{2-})
758	δ (D-ring)	755	δ (G-ring)	873	dGMP
738	δ (G-ring)	753	ν (PO_3^{2-})	859	CPZ
737	ν (PO_3^{2-})	720	δ (G-ring)	853	CPZ
688	δ (G-ring)	704	δ (G-ring)	803	ν(C-O-PO_3^{2-})
678	δ (N(G)-C(D))	664	δ (O-PO_3)	799	ν(C-O-PO_3^{2-})
645	ν (O-PO_3)	649	δ (G-ring)	783	dGMP
631	δ (D-OH)	631	δ (D-OH)	783	CPZ
628	δ (G-ring)	618	δ (D-ring)	775	CPZ
620	δ (G-ring)	605	δ (G-NH_2)	770	dGMP
612	δ (D-OH)	600	δ (G-ring)	767	dGMP
592	δ (G-ring)	576	δ (G-NH)	747	CPZ
558	δ (D-ring)	558	δ (D-ring)	744	dGMP
545	δ (G-ring)	523	δ (G-ring)	735	dGMP

a) D: 2-deoxy-D-ribose ring; G: guanine ring; δ : deformation; ν: stretching vibration.

were 1.401-1.404 Å, (Figure 1). The optimized bond lengths of C9a-N10 in gas and in water phases were 1.448 and 1.450 Å, respectively. The lengths of N10-C10a in the both phases were and 1.447 Å (Figure 1). The bond lengths of C4a-S5 in gas and in water phases were 1.755 and 1.758 Å, respectively. The lengths of S5-C5a in two phases were 1.755 and 1.759 Å, respectively (Figure 1). Second, on the net atomic charges of CPZ in both gas and water phases, N and S possessed their positive charges and the charges of N (0.097) and S (0.203) in gas phase were higher than those (0.075 and 0.133, respectively) in water phase. Interestingly, all calculated C-charges were negative with the lowest charge (- 0.215) of C4a in gas phase and that (-0.239) of C5a in water phase (Figure 1).

The infrared (IR) spectra of various phenothiazines have been measured (7). Using the measured IR spectra of CPZ and its derivatives, the CPZ aromatic ring exhibited a weak band at 3120 cm^{-1} (=CH aromatic stretching vibration), a triplet band at 1587, 1563 and 1493 cm^{-1} (C=C skeletal *in-plane* vibrations (7). Bearing in mind these known data, the normal coordinate analysis and assignment of CPZ in the

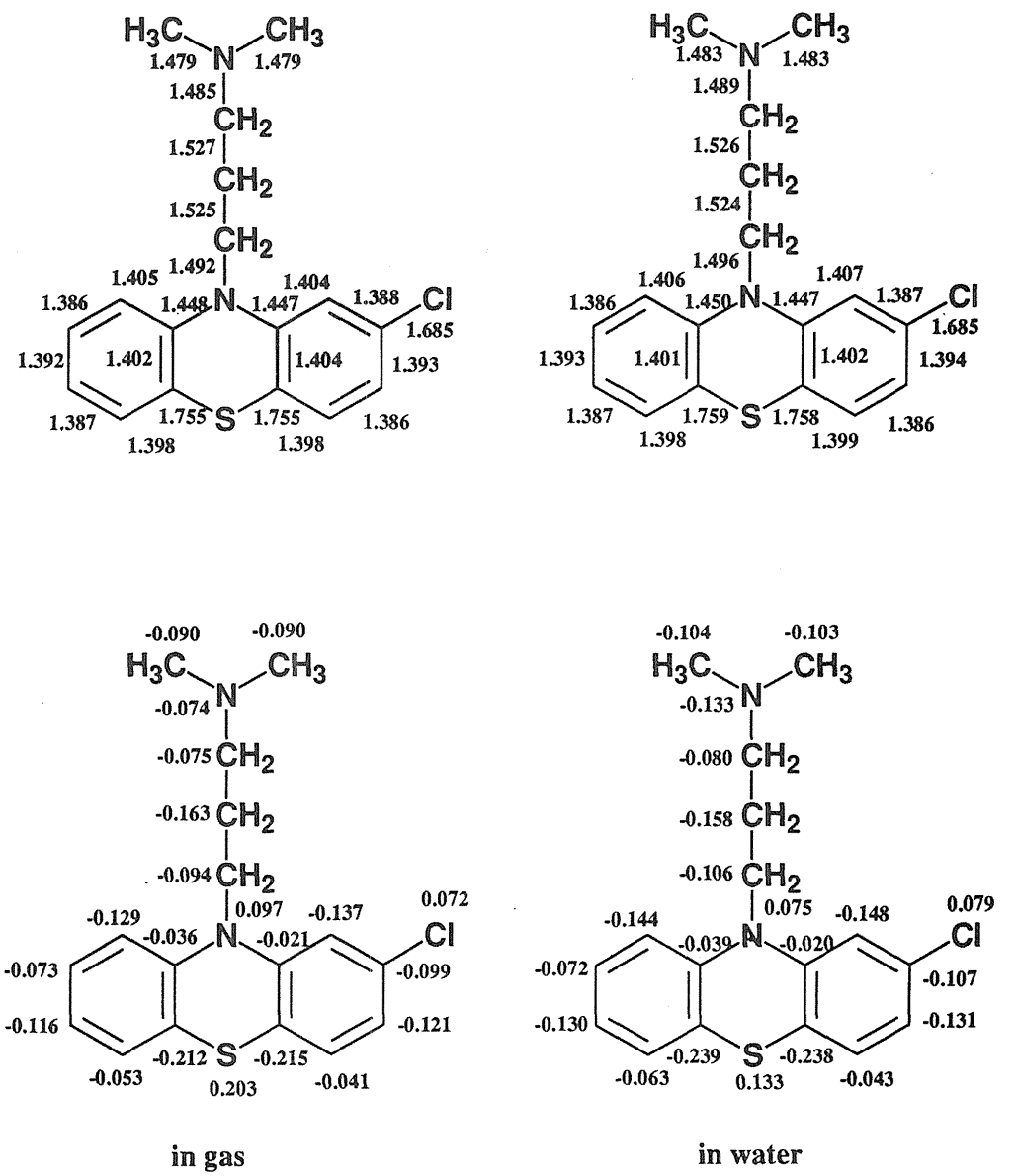

Figure 1. *Optimized bond lengths (above) and net atomic charges (below) of CPZ in gas and water phases.*

CPZ-dGMP complex by Force calculation ranging from 3150 to 1700 cm^{-1} were performed here (Table I).

First, the IR spectra of CPZ in gas phase, two main bands at 1783 cm^{-1} (transition dipole (T); T=6.00) and 1770 cm^{-1} (T=2.07) were assigned to two C=C skeletal *in-plane* vibrations of ν (C8=C9) + ν (C5a-C6). Second, in water phase, the strongest band at 2287 cm^{-1} (T=3.79) was assigned to two C=C skeletal *in-plane* vibrations of ν (C6=C7) + ν (C9-C9a) and second strong band at 1783 cm^{-1} (T=1.00) was assigned to two double C=C skeletal *in- plane* vibrations of ν (C1=C2) + ν (C3-C4) (Table I). The calculated normal vibrations by Force calculation were higher than the measured vibrations of CPZ alone.

Electronic structure of dGMP. For the low energy conformations of dGMP in COSMO and gaseous phase, the total energy as function of dihedral angles at C2'-C1'- N9-C8(τ) of dGMP was calculated by scanning by 10° τ, ranging -180° to +180° (Figures 2, 3, 4). From the calculated results, the corresponding dGMP of B-DNA and dGMP of A- or Z-DNA in gas and water phases had each two minimum total energies by the rotation of dihedral angle (Figure 3). We concluded that one of two significant calculated minimum changes in gas phase means that dianions of phosphate group are approaching a proton of the amino group of C2 amino group of guanine, and in dGMP the dihedral angles of C2'-C1'-N9-C8 were -60° and +130° for dGMP of B-DNA and

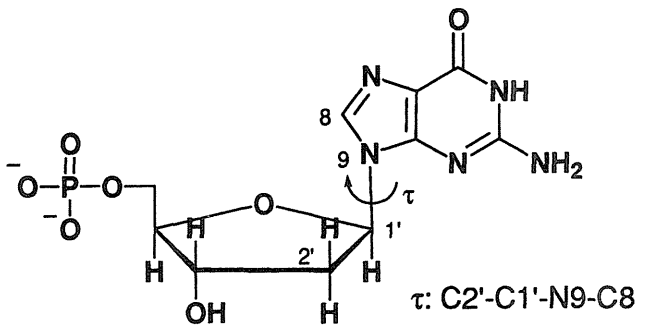

Figure 2. *A structure for dihedral angles at C2'-C1' (2-deoxy-D- ribose)-N9-C8 (guanine) (τ) of dGMP was calculated by scanning of 10° τ in the range (-180°)-(+180°).*

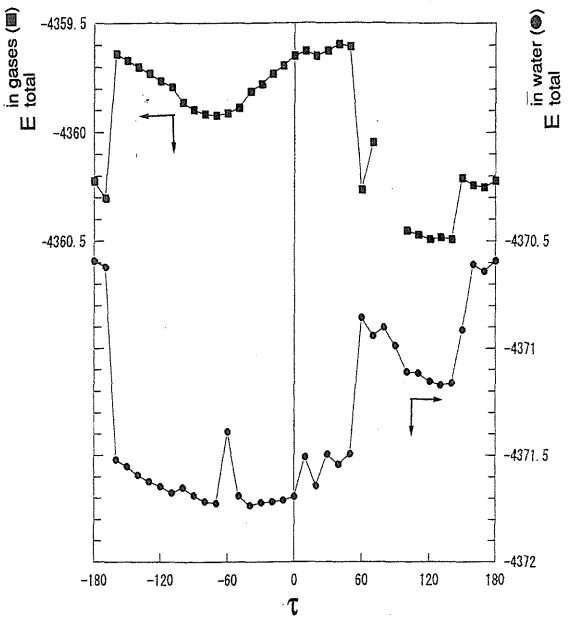

Figure 3. *Profiled energy (E:eV) of the rotation (τ: C2'-C1'-N9-C8) around the C1'-N bond for dGMP dianion by PM3 method.*

dGMP of Z-DNA, respectively (Figure 3). The structures from the calculated total energy (E: eV) with dihedral angle (τ) of the rotation of C2'-C1'-N9-C8 around the C(1')-N bond for dGMP dianion by PM3 method was proposed in Figure 4. Thus, both dGMP of B-DNA and dGMP of Z-DNA should take the lowest energy on the two corresponding angles in dGMP of B-DNA and dGMP of Z-DNA for the stereochemically highest stability of the coordination. The large difference of torsion angles of two components (guanine and 2-deoxy-D-ribose) in dGMP let the two compounds to stereochemically approach a dianion phosphate group and a proton of C2 amino group in guanine (Figure 4).

Interaction of CPZ with dGMP of Z-DNA bY PM3 calculation. The normal coordination analysis and assignments of dGMP of B-DNA, dGMP of Z-DNA and CPZ-dGMP systems were calculated by PM3 method (Table II). First, on dGMP of B-DNA, one vibration at 737 cm^{-1} was assigned to the symmetric P-O streching vibration of PO_3^{2-} group and two vibrations at 835 and 800 cm^{-1} were assigned to the anti-symmetric P-O streching vibrations of PO_3^{2-}. Second, on dGMP of Z-DNA, three corresponding vibrations in dGMP of Z-DNA to three vibrations at 737, 835 and 800 cm^{-1} in dGMP of B-DNA represented at 753, 834 and 806 cm^{-1},

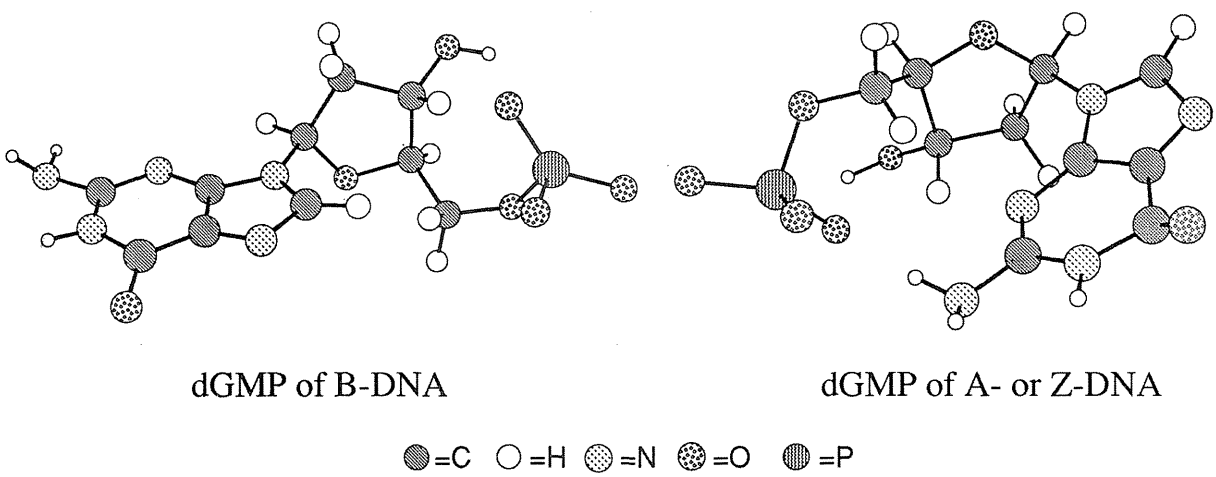

Figure 4. *Two optimized dianion structures of two type's dGMPs by dGMP of B-DNA and dGMP of A- or Z-DNA.*

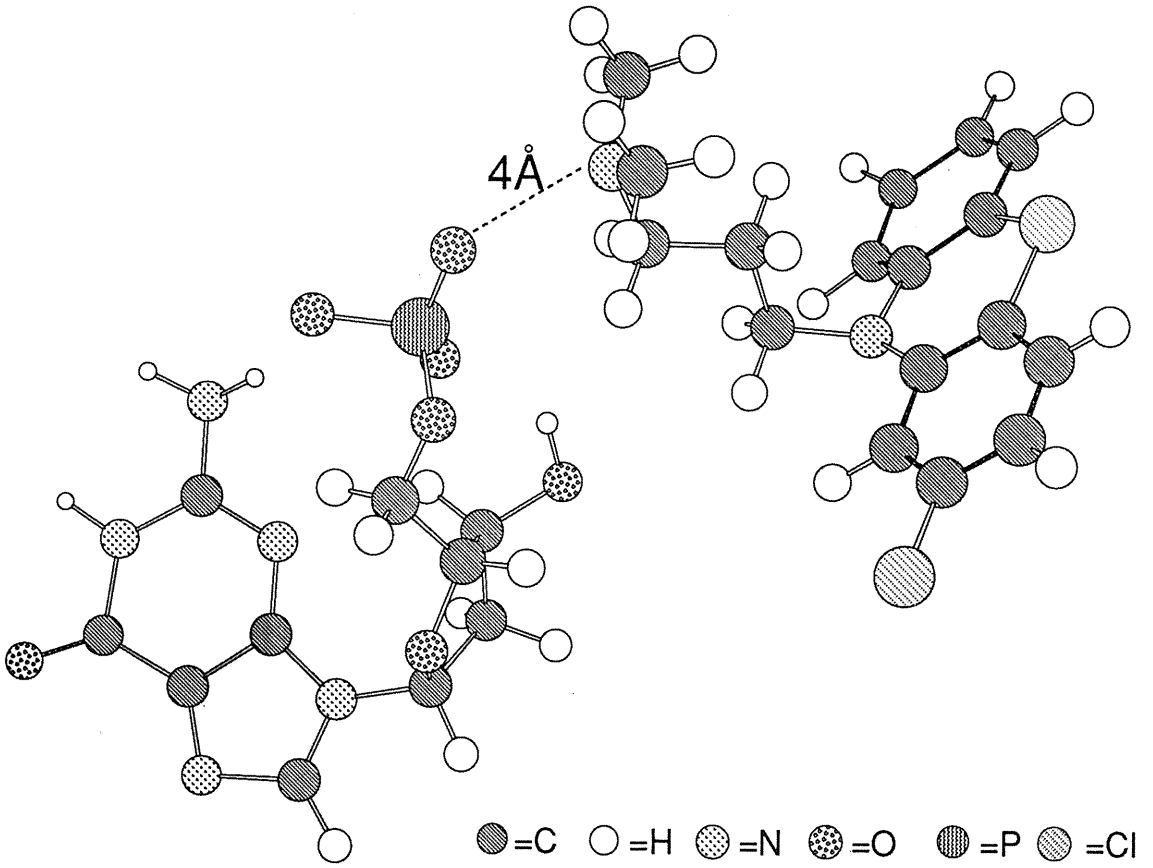

Figure 5. *Interaction model of CPZ with dGMP of Z-DNA.*

respectively. Finally, on a CPZ-dGMP of Z-DNA system, three vibrations at 889, 803 and 799 cm^{-1} have been assigned to the C5'-O-PO_3^{2-} group (Table II). The fact could be supported by the change of the symmeric P-O streching vibration of the PO_3^{2-} group in Raman and infrared spectra of CPZ-dGMP system (4).

It could be concluded that a oxygen atom of phosphate group on the dGMP of Z-DNA intercalated with a nitrogen atom of dimethylaminopropyl group on CPZ and an intercalating complex was formed with 4.0Å (Figure 5).

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